Dr. Kumar Prabhas, Dr. Maithri Basu, Dr. Vinish Shah, Dr. Pratik, Dr. Chakor Vohra, Dr. Anup Tarshniwal, Dr. Sunil and Dr. Nalada.
Okay, please come.
I think after listening to so many high-fi...
After listening to so many high-fi, you know, things about that, let's come back to earth. You want to come? Dr. Pratik, come.
My wife is because of the excession she has to be worse.
So, anytime that we talk about liquid by up, I mean normally the patients, first actions actually are like wow.
You know, I mean, where do you do this type of work? Why didn't you come before?
I guess, but my main impression is subsequent reaction to that after the report actually comes, just like, Rukhav, just have a sabar.
Let's just put this show test, you know, let us actually see whether liquid by-ops is really the stuff that is made to be.
So, we'll start from, come again.
Thank you.
I don't know whether I take it as a compliment or not, you know. I mean, the science is never taking as a compliment because science is where there is.
Entertainment is where here is. Okay, we'll start on this side. Just a yes or no.
Are you a believer in liquid by-ops? And I'll put you to test.
Just a yes or no, no, if sent but send everything. The mode number of time that you are taking out of it makes me say no.
Not yet. Not yet. No, yes, no, not yet. Fantastic.
Very strong believer.
I'm a strong believer.
Two is to one.
Three is to one.
You tell me, what is the problem?
You tell me, this is the first thing, I'm talking here, I'm sitting here, you are a believer, you are a hunger.
Okay, four is to one believer, five is to one, six is to one believer. Okay, I am not yet actually, I'm actually not yet over there.
Okay, so let me just ask from this side of the app, do you think liquid by-ops is a mean actor?
Oh, by the way, this is a Hindi, not a Hindi movie, Kanana movie.
I will just call it a Bhaubali or Telugu movie. You know, it's a Bollywood, this happened in Hindi and Kanah, this is a happened in English.
So tell the English people, no. Okay.
Or you think this is like Kata Pa, who is going to actually kill tissue by-ops, on a supporting role.
So what do you think liquid by-ops is, main role is going to be?
It can be both.
It can be both. Fantastic.
Doctor Pratik.
It's more of supporting role as yet.
Doctor Sunil, compliment, different task.
So I think everybody saw the slides, this is going to be the winning combo.
I think the tissue and liquid by-ops is going to be the combo is really, which is really going to make a difference.
So just a basic things about liquid by-ops, this is a slide which I got from last semester.
Which basically says, liquid by-ops is like fishing and not fishing for Goa, where Doctor Sahooh is going.
Already gone, already reached to the line there.
So catching the fishes when they are the fish in the sea.
But if you don't catch a fish in the sea, that doesn't mean that there is no fish in the sea.
Correct? So just because you are not able to get anything in liquid by-ops, it does not mean that mutation is not there.

Fares statement? Fantastic.
When there are more fishes over there, you are more likely to catch something.
Fares statement? However, you can use a single gene testing, you can use PCR or a DDPCR or RTPCR, a single call, but you can do an NGS.
So just a basic, I mean this is for this audience, this is very basic, but for me, this took a lot of time and then I will come up here.
So this is the basic rules that we actually have.
So what does the liquid by-opsie actually analyze? We will start from you.
So for you, does it analyze? You know, there are so many things.
It can analyze the CTC, it can analyze the CTDNA, CFDNA, proteins, TAAs, microvarin, Metabolites, DNA methylation or CTDNA.
What do you think actually does analyze and what it should analyze?
So they can analyze almost everything.
Correct? In the clinical use, what is being analyzed?
So what we need is the mutations, the particular DNA and RNA mutations is what we have to do.
The originality of the alteration is what we are interested in as clinician.
Okay, so Dr. Kumar, I will come to this so you would directly.
So I mean, you know, as far as I am concerned, this is my way of this, you know, not my problem, you know, Sarnu ki.
I mean, as long as you give it from CTC or a CTDNA or a exosome or whatever, TEP, it is not my problems, you know.
So this is the clinicians guide to a success.
As long as you give us a mutational load, whether it is CFDNA, CTDNA but from you and then from the lab people,
what is it that actually will give us the right answer for that?
So at this moment, it is CTDNA but looks like you will get a whole lot of information near future.
Because now for example, people are not able to get a cell out of here and able to sequence.
Now this issue will come up that think about tissue was, so tissue was negative.
CTDNA positive, we are treating today.
We thought this, you know, 10 years back we were thinking liquid biopsy, you will miss always miss.
Correct, we started looking, you may pick.
Now think about liquid biopsy negative, tissue negative.
And with a single cell, it is positive.
Yes.
So as on date, we are not there but yes, kind of technology, we are getting now, you know,
you will see, soon many things coming there.
So maybe we can follow Jain them, go her.
Okay.
As a friend, as a clinician, I mean should we actually be looking at what is the source of that,
it is a CFDNA or a CTC or you know TEPs or whatever or we just as a route over the Sanu Ki.
As long as you get some delineating positive, wherever it comes up, it is okay.
See, there is an alternative issue there.
No, we should look, you have a stake there.
You know, because the activate patient will be catching you.
Patient will always catch us.
Patient will always be there, then we will be there.
So, I guess that technology, no, someone gives a report.
You need to know that report.
So how confident you are with the person that he has gone through that technology part?
Because that technology is not easy.
But in case someone is very good in it and he or she gives it, today I don't be

routinely.
But you might just...
Thought.
I know, I'll say, hey, if I get Al positive third line, think about it or EJFR positive third line,
where everything is failed, someone say, I say single cell, I saw it.
Why use it?
I may end up using it.
But today I am not saying, please do single cell, no, no, that's not the message I am giving.
Today it is CTNA, but what's out?
In next, you will see, next few years, you will see whole lot of data coming from this fantastic.
So now let us talk about this is what I asked Dr. Anuraza at a message at 10 o'clock in the night, two days back,
when I was actually trying to prepare for this, I said, what do you want from me?
You know, even if you see CTC, CFDNA and BioMakas and lung cancer, I had no dash idea
about what exactly I was going to do with it.
So when in doubt, confuse more people.
So let me talk to Dr. Rapparna.
Not Rapparna.
Do you agree with this?
CTC versus CFDNA, one is a bride and one is a bride made.
Do you think CTCs are always related to their and CFDness, stick in the...
At least part of the bridal party for sure, the bride is different, right?
Do you think circulating tumours, so you have to actually understand what is circulating tumours?
Yes, that's what I want to understand.
Okay, sorry.
That is why I made fancy diagrams like this, so that you don't ask me all these questions.
I'm confused, but once I...
I know.
When you convince them, confuse them.
So a single cell, a single cancer cell, right?
Which gets funded tumoursite when a single intact cell gets liced.
That is your circulating tumoursil.
Okay.
CFDNA is self-readiene, is essentially from the blood sample,
when you take a blood sample from a patient, there are different components to it.
So in that, you will have the patient's DNA, which is a germline component,
you will have the chip mutations in it, you will also have your circulating tumour DNA,
which is essentially from the tumoursil, the somatic DNA that gets liced into the blood.
So the self-readiene has a component of all of this,
and it's how you extract it is what you're looking at.
So what is the problem with CTCs and Dr Pratik?
What is the problem with CTCs?
I mean, why is it why Dr Pratik Kumar sir?
You know, CTCs, CFDNAs, they are asian and CTCs, not really there.
Why is it that so?
So there are several technical limitations.
One of the prominent ones which we can cite and what we understand is of today is that
lung cancer is tightly regulated.
It's not very easily accessible for cells, as well as self-readiene to enter into the blood stream.

So what we are looking at is a large proportion of the patients,
which will be lacking these biomarkers, and whether third will be allowing us to generate enough of the clinical,
the externally, you know, outcome.
So that's a big question, but wherever it's present, there it will be reliable.
Okay. Anybody else out of Kumar, others on Elias first?
Basically, it's a sensitivity that is compromised in the CTCs.
The sensitivity is quite low.
Okay. The Kumar.
You know, one of the things we forget,
if you take an ACC in a few years back, before they ever, you know, recommended CTC DNA.
There was CTC.
CTC was there.
Everywhere was there.
There was a long process.
Everywhere there was there.
I tell you, there was a good conversation and we did have it.
And we had for years.
So when the CTC came and Jainth had a key interest on CTC.
So I don't know, whenever I saw the data, it was already in guideline.
And then he used to come to come to see this guideline and he was developing the technology.
I thought technology is difficult.
I thought, I don't know, he will be able to develop or not.
It's a different matter that he only developed it.
He got regulatory approval.
But I was always skeptical that CTC, you know, the way it looks like once,
I'll tell, three, three, if you miss two of them, it is gone.
But somehow, you know, CTC faded away.
So I should tease him.
That's not a chance.
That is exactly what I want to know is, what is the problem in CTC?
So it's not a story, it's not a story.
It's not a story, it's not a story.
Let me go to Dr. Jainth.
Yes.
Then as you tease him, I kept telling you, you know, this is not useful.
And you kept working on it, I kept saying that work on CTC DNR.
So he had finally, he considered, now it's the other way around.
Now when the CTC data for sequencing starts coming, now it is a totally different ball game.
Then he realized he was smarter.
He was smarter.
Fantastic.
I mean, we are dumb by the way, by the way, at least I am dumb.
This much I can realize.
After learning to CTC and CTC, yes.
Everything is written in blood.
Correct.
Goes around, comes around.
So I think they have different roles.
I would disagree to this panel that CTC has its own different roles because it's going to cause a next level of vertices,
which we ignore often.
So diagnostication is important from the CTC DNR.
That is well established.
If you have to monitor, which is most important as, you know, facet of treatment longitudinal.

CTC's presence actually shows that the progression is there in spite of no...
So can I just ask you a very simple question.
Let us say, if I have to diagnose a patient, CTC DNR is good enough.
Yes.
But for monitoring the patient, we have got a lot of CTC DNR studies also.
That is not good enough.
CTC is better.
Is that right?
Yes.
So you said it.
Okay, but that is not something which I read.
Actually Kumar, will you agree with that statement?
But you said just, you know, they can do us...
The spatial transcript or something, single set sequencing on that CTC.
Yes.
Why does it be better?
So, yes.
See, especially you will be better on CTC.
As you can see, it has your CTC DNR.
So, trust him.
I assure you, more I have interacted with him.
I have realized that he'll futuristically...
So I will say today, today, CTC DNR, but futuristically, five days down the line, you will have a whole lot of things related to CTC, you know, which will be there in, you know, use and multiple.
Yes.
So, monitoring the CTC DNR is better.
That is very easy.
Very easy.
No, I mean, there are a lot of papers.
I mean, that is what I was just...
There are a lot of papers which I can show you also.
Conclusion.
The point is, CTC is better.
That is what I want to know.
It is a twofold.
I mean, CTC DNR definitely is a monitoring tool.
If you look at a co-hend, at all that published, okay.
But I think the main cause of a metastasis,
seeding is going to happen from the CTCs.
So, presence of CTC is going to extend the disease.
Absence of CTC DNR, that means, yes, the patient is in the remission.
Presence of the CTCs.
What the MRD by NLR, the CTC.
Yes, so, cellular disease, which is going to cause an extra vegetation.
It is going to cause an invasion.
Okay.
I think there is a technology difference, you know, okay.
Doctor, gohar, what does it?
Group positives are more reliable.
Okay.
But the whole state is, that will be a bit of a change.
Because of CTC, there is a shorter, half-light in the blood.
So, that has a more reliable.
Okay.
Doctor, gohar, you have a point.
So, CTC is better than CTC.
No, that's what you want to say.
It's not the opposite.

But this one.
CTC DNR is coming from a dime-sense.
Okay.
We know that cells are dying and they are not important for monitoring.
So, as CTC is, these are live-wounds.
And they are going into the circulation.
We are going for a more informed, now we know.
Now, we are seeing molecular and exordinal diseases.
They are PDL1.
They are going really more over the surface markers.
Okay.
Example PDL1, not, you know, it's not as we know it on D-Shopar,
for a minute or 30.
But PDL1 on the CTC, they use their license to get the immune system.
They go into circulation.
And they go into the different parts of the body.
And, you know, the stress.
And second thing is, when we talk about C-A-N-E, you know,
we have not a, we should like chip variation of the visual.
So, when you do the CTC based, the chip will be out.
So, you can eliminate the chips, yes.
You can eliminate lot of noise, which will go through the aging process.
But one more point which I read was regarding the detection and enrichment of CTCs.
You know, the technology to isolate CTCs and enrich them is slightly difficult.
Is, has that been taken care of, Dr. Chen?
Yes, I think.
You know, we are talking about an old wine, when there is a new bottle actually
coming.
I think that is what damage that SOC study has done.
They are talking about enumeration number.
No.
One single CTC with PDL1 marker can deactivate T cells against it.
It is proven now.
And, as Dr. Kumar said.
So, at present, CTC DNA is okay for diagnosis and detection.
CTCs may be used to follow up.
The presence of CTC will be there more for MRD.
CTC DNA can be used to follow up.
Is that okay?
Fantastic.
Now, let us go to the basics.
Don't mind, but there are a lot of molecular people over here.
And everything is over here.
The status of companies which offer liquid by optis like this.
You know, and everybody says, I am the best.
I am the best.
I am our Chohatar, Jee Nee, our Patchar, Jee Nee, our Dosa Jee Nee.
We will give in 3 days, we will give in 2 days.
So, I have trust issues.
I have lot of civil trust issues.
And I think all the clinicians have.
So, I have written over here because Dr. Mehta will be here.
So, I wrote what all should have poor oncologists like us,
not oncologists like us, look in a proper map you have not spoken to now.
In a liquid by optis report, can you give me a Kunji?
You know, that is how I pass my pathology and microbiology.
So, look for what exactly suppose to be looked in a liquid by optis report.
But, you cannot do anything.
You will not be able to do anything.

What I know from that is from the theoretical knowledge because in our lab,
we have not yet started the liquid biopsy thing.
Great, thank you.
We are only working on the solid tumor.
But, yes, some basic parameters are very important in the liquid biopsy reports.
Like the depth, depth of the sequencing depth, that is very important.
And like this, multiple times what is discussed, the cheap nutrition,
what is coming or not, whether it is there or not.
These two is very important.
If you see the number of CTC, par, ml of plasma, that is very important.
Okay.
Let me go to Dr. Kumar.
From a clinician's point of view, just some five basic points that we should say,
you know, when we look at the liquid biopsy report and how do we trust that report?
See, at this moment, I totally agree with you.
This is one of the challenges.
So, what I do is, I ask every lab today also.
That show me some few hundred patients, data you have done in tissue and blood.
That is the first step.
So, one thing is concordance.
Concordance and especially for fusion.
Okay.
Because, see, you know, you know, you know, you know, you know, you know, you know,
testing.
So, it is DNA based.
And DNA based, you know, and the institutions.
Infusion.
Which is okay.
You know, and I need to, you know, consider that, I have been shown more than once
that DNA based,
you can do as good as you do with RNA based.
It is not like you will not.
But then also, and then it should be fusion.
And then some of the fusion is a tricky one.
Like for example, anti-RK.
So, it is a tricky one.
So, I just want you to see the raw data,
concordance and...
You have shown me, you have shown me,
that you have done the patient in tissue,
you have done the patient in blood, what was that?
If someone shows me,
all 10, I picked up an tissue and blood.
So, all 10, all 10 are...
I was not able to see the body in the same way.
I agree with you.
So, 7 shows me, 8.
Or 7.
You are comfortable.
So, you are now you are telling 7 on 10 is good, 10 on 10 is bad.
10 on 10 is bad.
They all are listening.
No, they show 7 on 10.
I agree with you.
I mean, we are smart people.
Sir, we will know only by 7.
I heard two things today.
One is to say, sorry, you will be okay.
So, what is 7 on 10?

You will be okay.
So, 7 on 10.
Some know, sir.
I mean, I am being honest with you.
People will not show the rear.
No, sir.
That's what...
Now, good thing is that, that multiple discussion,
now it has happened that,
there are lapsi, we have done it.
Please show it.
Okay, fine.
They have shown, sir.
7 on 10 is there.
Then the report comes.
Then what happened?
Then what all should we look for?
So, that's the first step.
Next step is, you know,
what you are doing,
anyway, other molotrotesting.
Hope they don't change the way of doing it.
Because, sure, as she was saying,
now, what was the quantity of DNA being put?
It will be less.
What you have?
About every reporting.
I mean I don't know,
ah, how much DNA was extracted,
عاما, nanogram of DNA,
I mean,
begitu.
And are we all doing it?
Or are we falling because...
What about what?
But, wireless nod □□te done.
So that is because, you know,
you are not asking,
I have nothing...
der Improvement of asking Chinese tomorrow,
and we are not asking these prices...
don't listen to them.
I don't, I am not asking them.
Listen to them from the diagram.
From have to be marketing then?
So there are laps,
20 pp. report say he ek page me let us have these quality parameters which we do what is the depth I know what much in fact you can ask what percentage of the sequencing
data you have not used in analysis because there are 25 30 percent of the data there
are people there first martyrs Janth, Sir is there Ramshree you did not analyze you have 20 thousand x think about they say 1000 x it is not 1000 x it is ultimately 700
I have a request.
It is good to have that nuance in a one page and one page will be good enough.
So rather than 20 page report, one page report, drugs, we can see for the last and we have been waiting for the drug.
He has not bothered about the drugs but it is a request from us to everyone because

sometimes you get, see everybody will come and say you know 100 WhatsApp messages will come, so we are the best, we are the best.
And finally when you look at the report there is nothing in the report.
So I think there needs to be a signerization among the labs and you guys will do a much,
much better job than with video, no anything.
We are just asking simple questions.
So Amali is an, we call Om Shrinath Om.
We call not a Mehta No, Malibu is an, but there are people everything around over there, that is the problem.
So not everybody has an institute, so we need to, you know, have a malgat tumor board then we have.
Okay, so just a last question over here, I will go to you.
Let us say you have a, when do you send a liquid by OPC?
And diagnosis, let us say tissue by non-smoker female comes to you.
A non-smoker female comes to you.
Your tissue by OPC report is showing no actual mutation.
Do you send liquid by OPC upfront or you send after that or you are happy with the tissue
in, just any, don't send a liquid by OPC.
So if I have good tissue by OPC, sample and distinctly, then I don't send the liquid.
You don't send, I don't report it.
Anybody else who will want to send a complete, on the science, forget the money of that.
I mean, Dr Amit, what will your, your take on that be?
I think there is complimentary.
Complementary will be something which is there.
Yes, there is a misnomeraliyat.
Okay, so this is what the, the Sanjay Popat study which I was talking about.
This study actually did a concordance between a tissue and a liquid by OPC.
And what they basically found out was that liquid actually missed less.
A tissue actually missed more and if you do both tissue and liquid 33% more samples were detected by adding both.
Timing of the test because timing is everything.
A complimentary approach is something which is very, very important but how many people are able to do,
very few people are able to do and that is predominantly because of the cost issues.
I mean, and you should be looting in dollars to actually get that approach from here.
So even the incident guidelines basically recommend that you can send both.
It actually says that we are 7-7 but that is an old movie.
It doesn't really work in today's thing.
So let's say you get a mate X on 14 positive on tissue, on only liquid.
Tissue means it.
How confident are you to give cap mat in about, at the bottom?
So I will discuss with the molecular pathologist.
They say they are the best. Yes, it is there sir.
And you call up the NGS paper also they say yes it is not there.
Then I will give it.
Then why do you want to call up?
I will tell you.
Come on.
I have to place the owner somebody shoulder and no.
Discuss with Dr. Sonil, he is very calm and still wins the give it.
I get.
So see we have been doing for EGF right now.
Only problem is the matter is so uncommon that we encounter one.

Yes, that is a challenge.
So I think so.
And this is where you know spending time with our multiple pathologists becomes important.
They will show you.
So whenever I have asked people this is negative and then you see it is there.
Where they manually check and they say it is there.
I would have IG.
I would have IG.
These are words.
They should use it to make you sir look good and you know.
But that when they show it then you know.
An agraripi.
EGF are used to doubt it when they started.
Yes.
But now we learn later on that EGF are used many of them in tissue and you pick up extra blood.
And you are treating it.
So.
Yep. Go ahead.
So this was for the vision.
Yes, please.
I just want to add here.
I don't think it is a different between the genes on liquid biopsy.
It is the nature of the test and the the way we look at the mutations like IGB was mentioned.
So it is not about EGF are is more confident.
No, I think it just came over there because it is a real mutation.
It is mostly medicine and you miss the bus.
So that is just like that.
Exactly.
And also because I had a study on met that is why Dr. Methasad.
So the problem is that.
In this in the tissue biopsy you are looking at a particular BF2 report.
Yes.
The laboratory has decided that it will use 5% BF.
Correct.
And it and if the laboratory has done the due diligence it will show it on the RNA as well as on the DNA.
So that is about the solid.
In the liquid you don't have these hurdles.
In the liquid you just say it has a fractional abundance of points in the body.
And then leave it to the nation to the nation to the etogin DNA.
So this cuts both ways.
You want to have certain BF for the solid so that you give appropriate treatment and you don't the fall school are not included.
The another problem with met X114 is that it has a pseudo lock and a homologue.
And it often it often aligns with PPKR1.
So on an IGV browser you will see several times the call has been made with deletions.
You see it?
Now what do you do with it?
It is a very difficult call to make when you have that kind of a IGV appearance.
So don't put them in that comfort zone.
That you will see the browser and make them.
No no but sir then what do we do?
So here there is a true challenge.
The challenge is that you should use an RNA and a DNA both.
And you should show complete entry alteration in both.

And both the ones.
Until that happens you wait.
Sir in the IGV I will not say this let's get the right date with.
The idea was that if you have an in the realignment program running in the pipeline.
Which will take care of this misalignment.
Will they still call?
No sir with the endelry alignment we get rid of.
So the call has been made after appropriate penetration.
Happy plate.
So it has weight penalty for a deletion.
Two deletion, three deletions per one SME.
And it is still very difficult.
You see usually okay there is a deletion.
I will do a single gene confirmation.
I will also select you one.
No.
I think you will have to do a lot of due diligence and first everything.
Let's hear them out.
Okay.
No I have completed every bit you sir.
The only idea is that we are deletion.
Yes.
We need to understand more.
I have told you to do a short detour.
You call it the organiser.
I have to do it.
I will do it.
People will remember this discussion.
What will happen to me?
You will wonder your value for entertainment.
I have got this.
I am going to show you.
So physically?
Yes sir.
Yes sir.
What?
So I think there are two three points.
One is when we do the validation say 5% of the graph.
I saw it.
Let's imagine I got a graph of 4.5% should I discard it?
No.
I think what unratio say is it's about misalignment.
It's about misalignment because of the pseudogen.
First we when we talk about a graph you know do you have the cut off?
Yes.
And sometimes it comes below cut off.
We agree with that.
That they will write in the report also that the graph is less than 4.5% and then they will inform the clinician.
That happens all the time.
That's okay.
Tell them about the liquid bioxy.
We don't have liquid.
We don't have liquid.
We don't have liquid.
We have to do that.
I am reporting that's why it's by the graph.
That is okay sir.

Sir, it's tissue.
What do you do for a hypothetical or two-way chart?
Sir, correct sir.
It's liquid.
It's a graph.
Now we have to complete it.
It's specifically exon 14 which we talked about.
It was very nice discussion all three.
So we heard Dr. Mehta, maybe you want to say first or?
Yeah you can go.
So what for example tissue is negative?
Blood is positive.
So I let you put it for clinician.
When will you think it is false positive?
Yes.
Is it possible?
So yeah so there is a possibility you get a false positive which is...
How?
For us understand.
Yes.
It could be like 0.1% or 1%.
We know that there is a limit of detection.
There is a sensitivity of every essay.
But when say for example if I had to do this,
I found exon 14 mutation on DNA in liquid.
I'm not talking to RNA.
So RNA is not laid as far as we talk about because there are a lot of issues.
Yes.
So DNA now what is important is when you have designed the essay.
And if essay has been designed and you have captured those you know,
regions, spice regions, the probes are very well put there.
And the tiling of the probes is very important.
So in simple languages you know I use this if I have to...
If there is a one terrorist in the room and we send one army man to capture or kill that,
there is a 50% of chances we capture and kill.
Now if I send two the probability of capturing or killing them is increasing.
If I send 10 I increase 10 fold.
So that becomes very important you know when it comes to the designing of the essay.
So if essay has been designed in that way and then you capture it the probability of this
capture in the 14 mutation on DNA is very high.
And when it comes canonical, non-canonical you know annotations.
So what is happening often you know when we do the analysis,
it's very important you know when we do the analysis.
First we do the alignment of the reference genome and then we do re-alignment.
So why the re-alignment is nearly this to avoid you know if there is an insertion deletion in delts.
Because they are tricky you know you re-align to make sure that you know you have aligned your reads appropriately to their actual you know reference genome.
So that you avoid that false positives.
If I have not done re-alignment now any structural variant including supplies variant, translocations,
fusions, copy number are not accurate.
So I believe most of the band formations will be doing that.
You know that's the important step you do the re-alignment.
Now some people use online tool you know just to do something and we don't know what is there.

So now if I have a tool I just upload something.
What has been in the background we don't know that and then there is a higher probability of you know I miss it and it is a false negative.
So if the alignment is there and you see it you say it's positive then for it is not you know false positive is true positive.
Am I right?
In addition to that yes.
In addition to that there are multiple parameters like quality, base, quality.
Dr. Kumar I think we need to last for the last 30 seconds.
I agree with his explanation of indel re-alignment to further transfer it to you know more easy language.
In the first pass of alignment we use a first algorithm which is little bit less accurate.
In the realignment we use a secondary algorithm which is very accurate and that we are doing only in the region of mutation.
And that is allowing us to improve the accuracy.
If those two checks are there then I would say true positive call is a reliable.
So just in case none of you also understood a millimeter of what they were talking about you not alone.
I was also there along with you.
You know something I read about IJV and miss Alive and realignment world.
I will go and read it later.
And then with you but thank you thank you all the.
I am most of the points that I wanted to say has been said.
It's just that I want to re-emphasize that the padding, intron padding and the panel design is the most important thing.
When we look at the liquid any liquid by upsie as say because the padding and tiling what Dr. Kohar told that it is very important and then the quality.
Sir, today is not going to be that much.
So that is okay.
So thank you.
I think thank you.
I mean I could not have asked for a better this.
Thank you.
Thank you for a wonderful panel discussion.
Yeah, thank you.
And for sharing this session.
I think we quickly want to the last session panel discussion.
May I invite Dr. Paneeta as a chairperson.
I think and moderator is Dr. Thrupti Pai.
She is not.
She is not.
Thank you to complete this brilliant molecular workshop.
I invite Dr. Thrupti Pai, professor of molecular pathology.
That's correct sir.
Molecular pathologist at data memorial.
Dr. Thrupti.
Thank you.
What do I know?
Okay, so very good evening to all.
After the most controversial topic of CTDNA, CTCs and most entertaining panel.
This will be a little boring one.
But again my point is to highlight issues which we encounter regarding future analysis.
So today we have best of the panelists.
So I would like to invite Dr. Chakor Vohra.
He is not there.
Okay, Dr. Akhil Kapoor.
Dr. Amit please.

Dr. Om Sri Shetty.
Dr. Suryuchi Agarwal.
Dr. Kuna Sharma.
Dr. Anurag Mehta.
Dr. Anuradha madam.
Dr. Mohini Ka.
And Dr. Plathik.
So maybe.
Okay, so the clinicians, issues and challenges I think we have already covered.
But still I would like to ask the medical oncologist here like regarding this interpretation of NGO's reports.
What are the usual scenarios?
That difficulties you have.
Common difficulties.
The reports are not complete because it does not have many of the parameters which we want to look at.
The depth of coverage.
So sometimes the reports are inadequate, especially when the reports are not being discussed with the clinician.
So that is a very common problem.
But since we have most of the time in-house reporting that is not so much with us.
So regarding reading on your own.
What about you?
So important things are the tissue content initially.
So most reports they miss us.
Sometimes the mutations, right, they are multiple.
And which is significant, the weft really is one of the things.
So even with less, it has been written at the top.
And the reparative is real driver mutation or just a passenger mutation has nothing to do.
So we don't have that idea.
Sometimes we see the active people to 3 more than 50%.
Few mutations are like which are not a driver but still having a very high wear.
So all these things we obviously go with a molecular tumor board then re-discurs and then come back.
But there are many mutations which comes which are no more.
So with more and more comprehensive or broad-based, we have this issue of multiple variants.
And where there is a difficulty in interpretation.
You want to add something?
We have a problem with the variants of unknown significance in all weird genes which are reported.
And we don't know what kind of an interaction they have with the driver.
So that is one area where I find it very difficult to understand.
Again with the broad genes which are recently started, we will have lot of data but not literature much on it.
So this is the usual scenario that we have.
I will just skip three.
I will just directly go on to this case one.
This is a 57 year old male, no family history of cats or smoker and with complaints of cuffs since one month breathlessness.
And cities can there is a lung legion which is encasing vessels and all.
There are mediascinal lymph nodes.
Biopsy is there.
So Dr. Kunaan with this histomorphology and this content are you, do you want any further test?
Or you will directly run NGS or you will not?
No I think this looks like a poorly differentiated carcinoma.
I can't really see a high power view whether there is any characterization here.

Now it looks like there is some characterization.
There will be this EOP50, a single icing, a covered surprise and get our diagnosis in place.
And then the only subject to further molecular testing.
Okay so as we I saw the I showed you this course.
So there are sometimes there are multiple course but humor is very scanty.
So pay a waitress to the histology.
Like in this case there is a very typical caratinization.
So it's okay even if you don't do the IHC you can directly write that there is caratinization and you can call squamous and then leave it to the molecular.
Now in a case of spamous with the 57.
I would just like to say the only reason why maybe an IHC might help because you might find both components and you know
Dividing mutations for an atom component are different and the way we treat squamous.
Yes that is a very good point.
So look at the histology.
When you feel there is suspicious for an atom component then you run.
If throughout there is a same morphology with squamous there is no need to run IHC.
So with this there is a squamous carcinoma.
And so every case every I mean across the ages in squamous will you ask for molecular.
So elderly patient also.
So again a point to be noted that even a squamous carcinoma across all ages we go for upfront NGS.
So this was a little older case the EJFR was done which was negative and PDL1 was 20%.
So would you like to go for NGS?
So in current scenario yes NGS was done in this case.
And this is what is the report.
Okay so now looking at ALC are you happy like you have got alteration in ALC?
Yes so I will come to you.
I will just ask what is there Dr. Mohenika.
Are you happy to have this report?
So this is a very long specific panel 50G in panel and you have no other alteration you have ALC in balance.
I would like to know the the translocation what I said.
No this is the report there is no fusion anything you have got the report of ALC in balance.
Then I am not very sure what to do with this report then.
Okay so you will have noticed the molecular too.
Dr. Anurag sir.
So we have some cases like this where there was an imbalance by finally the balance was there.
So this is a supporting marker of a fusion rearrangement because as the as the translocation happens one part will be transcribed less the other part will be transcribed more so this imbalance will happen.
So in this case unfortunately you are not getting maybe you have not done the RNA sequencing together or you are relying on it.
So this is DNA RNA and just done both and this is the final report.
So if this is the imbalance then the fusion has happened and therefore there is an imbalance.
Why they cannot just write ALC fusion?
I mean this is not a fusion rearrangement.
They have not seen it but it is also a supporting element for a fusion rearrangement.
So when you are seeing a fusion rearrangement the certain values it must cross that value or it should be below that value then you then your call of a fusion rearrangement is more concrete more solid.

It is a supporting element but in this case you do not have the ALC on so do a fish or do a IHC and possibly they will come past it.
Okay Dr. Suryuchi and then Dr. Omshriya.
So basically there are different methods of doing ALC fusion.
So this is one of the methods actually I think they have done RNA but they have done a PCR based method.
There they are looking at the exonic expressions.
So exonic expression of the 3 prime end of ALC will be lower as compared to the 5 prime end of the ALC if the fusion has happened.
So this is probably this method that is where they are not able to report the partner.
When you do NGS-based RNA sequencing you will get the partner as well as and when it is DNA and RNA based both you will even get to know ALC imbalance.
Actually adding the confidence to the fusion that you have detected through RNA based sequencing.
So whenever we are ordering the test we should know the method what they are using.
So ALC imbalance is obviously confusing whether we should take it as a positive or not.
However it is expression based it shows that the half of the ALC is expressing more which may or may not contain kinase domain that also we have to know.
So RNA based NGS sequencing to detect the fusion will give you many more information like whether it is infrain fusion whether it retains the kinase domain.
Sorry to interrupt but this is RNA based NGS also.
I mean they have done DNA and RNA sequencing NGS and they have not found any fusion partners and this is the report after doing RNA based NGS.
That is very unusual it could be some out of frame or uncharacterized rearrangement that they have repeated as ALC imbalance.
So RNA based will give us definitely a partner retention of kinase domain or not partial domain or full domain plus unfraithful not always actually.
No I mean whether it is there or not at least we will get that.
So Dr. Umshti Shetty we also have this panel so this is some outside report but we do encounter this and this is this upper panel.
Sorry. So yeah I mean we see this trend even in the NGS panel which is Amplicon based library preparation.
So you do see I mean but then it is NGS which is spanning the.
So if it is hybrid capture then it will be much bigger.
So yeah agree to your point hybrid capture will be more specific it has its own pros and cons but Amplicon based library preparation on NGS spanning the break point regions with having several 5 prime 3 prime regions crossing across the tyrosine kinase domain.
You still see these things and usually in transcriptomics when you have a whole transcriptome RNA transcriptome this algorithm is extremely useful because you can go back and look into the entire data.
And NGS we know limited panel like OPA you do get these things so as rightly mentioned it is always good to go back and do an orthogonal testing and we have seen several instances where Alky imbalance was there and we didn't get it.
Yeah if you already made hybrid capture you not have to do any transformation.
Yes I will come to that library so point is that sometimes you have such cases where you don't have any fix answers coming to this panel.
And you do have right side partner and left side partner because this is Amplicon but this strategy is in OPA which is like a exon tiling again a different type of PCR which in the which will help us detect novel fusion partners.
So here it means that you don't have a typical partners that are present in the panel but you might have a novel fusion partner.
So that is what is this panel and so.
No I am just a little bit on the RNA sequence in case the test detection if you include the exons of all OPPO genes.
I mean all exons of an OPPO gene you will be able to pick up known unknown partners.

So if you know so this is an Amplicon base this is not a hybrid capture.
No exactly so Amplicon can because known unknown partners based on the expression of exon cd we have.
It can be more.
Yes there is an indirect observation of our fusion.
Yes.
And support to be called equally explain but some of the exons are highly afraid then other exons are lowly afraid.
There is an indirect indicator of a fusion happening.
Yes exactly.
It is a surrogate evidence.
Yes.
Okay.
Orthogonal testing was done and IFC is negative.
Let me now put across this. IFC is negative here adding to further confusion.
So we do encounter such cases.
The point of including this expression analysis is in general to identify novel partners where as Dr. Plathik rightly said it is an indirect evidence.
Now question to the medical oncologist are you ready to give now there is no other alteration nor the driver thing.
Are you ready to base your treatment based on this report?
Definitely not.
What was IFC also negative?
Yes yes IFC is also negative.
Fresh was also negative.
Yes.
Then we will take it as negative only.
Yes.
So sometimes we think NGS is the final answer but then again some cases we have to go back and switch over to some orthogonal testing and this is one such examples where lot of discussion kept on happening because whether to consider this or not.
So coming to this.
So challenges and solutions.
So that is what I wanted to tell what is the should Dr. Anurada.
Challenges and fusion detection.
Yeah.
When you are doing the actually the RNA fusion, you want to look more of fusion I think we should go with the hybrid capture method.
That is the best part to do it.
And in the fusion detection like in the liquid biopsy there can be a challenges in the fusion detection not in the tissue biopsy.
Anurak sir.
Okay you can come to hybrid capture versus amplic on base for the audience.
So I mean obviously hybrid capture requires higher input of DNA because you have to pick out that particular.
You have to you know bait it.
So you need both amount of DNA.
The amplic on base require less amount of DNA.
And if you have actually made the climbers pretty well, you have designed them well because one of the partners is always constant.
Break down the other partner has a variable with a break point.
So if you have designed properly, I think you should not miss them on an amplic one also.
But theoretically probably hybrid capture is a better one.
Coming to the real world life scenario, how many also Dr. Suruchi you were saying hybrid capture?
We all know hybrid capture is very good in terms of sensitivity.
So but when it comes to RNA, how frequently does all these engines, libraries are based on RNA?

I mean on based on hybrid capture.
How frequently like maximum lapse if you see RNA is amplic on base.
DNA is maximum time hybrid capture.
But because RNA have little poor quality, amplic on works better.
So which panel do you use?
We use hybrid.
We have all of our panels on hybrid capture.
So all functions on hybrid capture.
So what we do is we include the exonic regions.
So among RNA and DNA.
So we include the exonic regions of all the genes that we want to use in our panel.
And it can identify known as well as unknown partners.
Because if the probe is binding to say for example,
Alc exon 6 just an example.
And it is the break point is at gene A which is even not in the panel.
The half of the read will align to Alc and half of the read will go in align to that gene A.
And unknown partners will be picked up.
We have picked up lot of unknown partners, rare partners with Alc as well.
And we have seen the response as well.
Coming on to the liquid biopsy.
No, I will come to that.
So with hybrid capture you pick up unknown partner.
But just that the quality of nucleic acid needed is little higher.
So in diagnostic settings where you have more of referral quality,
poor tumor tissue is less.
More practical is amplic on.
But you have to keep in mind that you might miss on certain unknown fusion partners.
And also it will give more information as I was mentioning about the domain retention of the read.
And in frame and out frame kind of information also will be okay,
but not hybrid capture because entire class is very busy.
Okay.
Okay, we have just four minutes.
I have little important points to make.
Next discussion is on DNA based fusion and RNA based fusion.
Just two points like sir,
RNA based fusion how comfortable you are using.
Yes.
You did weight pair sequencing.
The regular sequencing will probably DNA based fusion rearrangement.
Seeing will be you will be have lots of false identity.
Okay.
Dr. Omshri Shetty.
I still feel I mean it's better to go for RNA based fusion,
rather than DNA based.
Of course in a scenario when you have tissue is an issue.
I mean at that time probably you can think of going for DNA based panel,
but you would end up missing.
Yeah.
So RNA is not bad because remember you have thousands copies of messenger RNA versus two copies of DNA.
So this concept that RNA yield will be less,
we have seen in our practice RNA yields are always better.
So it is more sensitive DNA missed.
Yes Dr. Kim, as a clean issue.
How reactions as compared to RNA rejections.
I am sorry.

We do say more of DNA rejections as compared to RNA rejections.
And that is because of expression.
Yes.
So what you call it the air force is here.
You don't have tissue material left over.
Would you still disrupt it?
No, no, no, no, not because some type means the electronic sequence will be too long.
It is covered by the PCRs.
How what even the largest length of a fragment that you can do PCR on a formal infid section?
No, no, no, no, no.
Generally for NGS the insert size varies from 75 to 100 to 30.
That, yes.
Isn't it true?
That is the most comfortable.
And I am telling you the METX on 14 is keeping the single gene acid that I have prepared.
The 235 fusion which is without escaping.
Clearly you see.
You only see the 94.
But you are happy seeing the 94 because that means the fusion there in it does happen.
Okay.
So there are two important points when we look at DNA based fusion.
One is the technicality, the probe design and the other one is interpretation.
So when you are doing hybrid capture based DNA using DNA as a fusion detection tool,
you have to actually include the intronic break points.
And if you do not include if it is unusual break point you will definitely miss it.
That is about the technicality of the probe design.
About the interpretation when you get the fusion event always happens at DNA level, right?
So fusion event has to be transferred into a meaningful transcript which is translating into the overexpression.
So if the fusion has happened in a way that it is rendering the transcript out of frame,
then obviously it will not.
So even if we see any event at DNA level, it may or may not transcribe into a viable RNA transcript.
Please remember 7280% of out of frame or frame shift, rear arrangements lead to nonsense mediated decay.
Yes.
So you will not get any messenger.
Yes. That is why.
Only 20% of the frame shift alteration which may actually get transcribed.
Yes.
So your lines on RNA for the sake of out of frame mutation is probably sort of very far fresh.
Just in some kind of a...
Agreed sir totally.
So whenever you are using DNA based panel for fusion, remember intronically regions are very large,
but you have to make it to sequences, your panel designing library should include those,
otherwise you will miss.
So there are chances of false positive as well as false negative with DNA based, RNA based are more sensitive.
So, I think just last but because I think I have...

DNA based, you can do mate pair in mate pair, you circleize, you circleize,
and then the two words come close and then you sequence the two words.
So mate pairs, you can do one with each one.
I couldn't do it.
Not the regular sequence.
Okay.
So with this, because Maya was stressing on this, the next session is waiting.
Okay.
Sorry.
Okay.
Just by I was highlighting this, because the liquid biopsy, CF DNA that you get,
mostly you get on DNA based fusion.
So, so fusion testing on liquid biopsies, so what is the cutoff?
Just last point on cutoffs.
If anyone can add DNA based, what is the cutoff and RNA based, what is the cutoff
for positive fusion?
In liquid biopsy.
So, so the panel I use, 17 nanogram of DNA, 30,000 depth, I can go up to 0.1 and I
will take 0.1.
Of what do you mean?
Of which?
Frictional abundance.
Because that's what the domogram has told me.
So, I am not stepping out of that domogram.
So, basically if we are using CF DNA for fusion detection, hybrid capture method,
we, if we see, we use UMI's.
So, if we see two to three reads also, and it is true in IGV,
and we do report it the fusion.
If it is actionable fusion.
CF DNA fusion, honestly we have not tried it yet.
I guess probably we are going to do, but whatever I have read from the literature,
I guess, van Reev is also okay, but humor fraction is extremely important and the
abundance.
Normally, we rely upon three molecular panels in a molecular family,
those which have developed from one pair of real months,
10, we are actually here, we can repeat it here, and we such that you should be
there.
That is what we use as a pattern, but in respect to, I will not be 0.01 or 0.01
because using that domogram, there is no more depth, particular depth,
the domogram is not good, DNA used, you can only go down to that much,
beyond that, they are thinly cutters.
Okay, sorry I couldn't even complete one case.
So this is how controversial RNA is.
So I am really sorry, thank you all panelists.
Thank you.